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# The number of multinucleated trophoblastic giant cells in the basal decidua is decreased in retained placenta

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## ABSTRACT

**Aims:** Retained placenta (RP) is a major cause of obstetric haemorrhage. The aim of the study was to obtain a better understanding of the mechanisms that cause some placentas to become retained, while most are not.

**Methods:** 23 RPs clinically diagnosed as placenta adhesiva and 10 control placentas (CPs) were examined for differences in trophoblast fusion into multinucleated trophoblastic giant cells (MTGCs), defects in the basal decidua, and decidual attachment of myometrial fibres.

**Results:** The number of MTGCs in the basal decidua was significantly smaller in RPs (0.23 MTGC/standard length) than in CPs (1.11 MTGC/standard length) ( $p < 0.001$ ). Defects in the decidua were observed in 4% of the RPs and in 0% of the CPs. Myometrial fibres were attached to the decidua in 78% of the RPs and in 0% of the CPs ( $p < 0.001$ ).

**Conclusions:** In placenta adhesiva compared with CPs, significantly less MTGCs were present in the basal decidua, the basal decidua was intact, and myometrial fibres were more frequently attached to the basal decidua. It is speculated that these findings may indicate that defective fusion of trophoblastic cells into MTGCs plays a causative role in placenta adhesiva.

Retained placenta (RP) is a major cause of postpartum haemorrhage<sup>1</sup> and maternal mortality worldwide.<sup>2</sup> Several degrees of adherence of the placenta to the uterine wall can be identified: placenta adhesiva, accreta, increta and percreta. The most common type of RP associated with abnormal placental adherence is placenta adhesiva (PA), with an incidence of 1.8% in nulliparous women.<sup>1</sup> PA is an abnormal adherence of the placenta to the uterine wall, with a clear separation plane found during manual removal of the placenta.<sup>3</sup> The mechanisms underlying abnormal placental adhesion in PA are not well understood.

Placenta accreta happens far more infrequently (in 0.014–0.3%<sup>4–6</sup>) than PA and has two definitions. The clinical definition of placenta accreta is that of an absent clear separation plane during manual removal, resulting in a difficult manual removal in pieces.<sup>4–7</sup> The histological definition of placenta accreta is that of defects in the basal decidua and myometrial fibres attached to the basal decidua.<sup>7–9</sup> Placenta accreta, as demonstrated by (partial) absence of basal decidua,<sup>7–9</sup> is associated with damage caused by prior uterine surgery, including caesarean section and curettage, placenta praevia

and advanced maternal age.<sup>5–6</sup> Such associations are not clear for PA.<sup>10–12</sup>

Inhibition of trophoblast invasion is essential for normal adhesion and expulsion of the human placenta after delivery of the infant. The apoptotic rate of invasive trophoblastic cells in term placentas is close to zero.<sup>13</sup> Frank and Kaufmann postulated that the main mechanism by which trophoblast cells lose their initial invasive character is syncytial fusion that results in their incorporation into multinucleated trophoblastic giant cells (MTGCs).<sup>14–16</sup> This concept was supported by the observation of relative absence of MTGCs in human postpartum hysterectomy specimens of placenta increta and percreta,<sup>9</sup> whereas normal placentas contain MTGCs in the basal decidua in 100% and in the myometrium in more than 50% of cases.<sup>13</sup>

The possible causes of PA, the most common form of RP, have not been studied in any detail. Analogous to the causes of placenta accreta, one might argue that relative absence of syncytial fusion or absence of basal decidua could play a causative role. Therefore, the aim of this study was to get a better understanding of the mechanisms underlying PA by determining the association of PA with (1) the number of MTGCs, (2) the presence of defects of the basal deciduas, and (3) the presence of attachment of myometrial fibres to the basal decidua, as a sign of abnormal adherence.

## MATERIALS AND METHODS

### Study group and controls

Twenty-three placentas clinically diagnosed as PA and 10 control placentas (CPs) were included in this study. The obstetric history of the study patients is shown in table 1. All women had an uncomplicated pregnancy and a gestational age of at least 37 weeks.

RP was defined as a placenta that had not been expelled at 60 min after the delivery of the infant, despite active management of the third stage with oxytocin, controlled cord traction and catheterisation of the urinary bladder.<sup>17</sup> PA was defined as a RP that, during manual removal, was adherent to the uterine wall without clinical signs of placenta accreta (ie, difficult removal of placenta in pieces). The CPs were expelled within 10 min after delivery of the infant. All patients gave their informed consent.

### Histology

The placentas were preserved in 4% buffered formalin. From each RP, four sections were taken.

**Table 1** Obstetric history of patients with retained and control placentas

	Control placentas	Retained placentas	p Value
No. of patients	10	23	
Nullipara	4	12	NS
Multipara	6	11	NS
Gestational age, weeks*	40.6 (37.9–43.2)	40.3 (37.3–42.3)	NS
Previous retained placenta, n (%)	0 (0)	5 (45†)	<0.05
Previous caesarean, n (%)	0 (0)	2 (18†)	NS
Previous curettage, n (%)	1 (10)	3 (13‡)	NS

\*Median (range); †Percentage in multiparous women; ‡Percentage in all women. NS, not significant.

Two sections were taken from the macroscopically damaged basal plate, because it has been shown previously that the junction between the intact and disrupted basal plate is the optimal sampling location for the detection of myometrial fibres.<sup>18</sup> In addition, two random full-thickness sections were taken, containing decidua basalis from the peripheral and central parenchyma. All CPs were macroscopically intact, and four random full thickness sections were taken from each.

The tissue samples were routinely processed and stained with haematoxylin and eosin. Two experienced observers (HvB and JB), blinded to the clinical data, examined the slides together and scored them in consensus. All slides were examined for MTGCs in the basal decidua, defects in the basal decidua, and the presence of myometrial fibres attached to the decidua. MTGCs were identified as large trophoblastic cells with voluminous cytoplasm and three or more nuclei.<sup>6–14</sup> MTGCs were counted only if positively identified as such by both observers. All MTGCs present in the full thickness of decidua basalis of the placenta were counted in 10 regions of standardised length using a graticule in one ocular of the microscope. The Merz graticule was projected over the decidua basalis of the placenta, and MTGCs were counted at magnification  $\times 200$ .

### Statistical methods

SPSS 10.0 (SPSS, Chicago, Illinois, USA) for Windows was used for statistical analysis. The non-parametric Mann–Whitney U test was used to test for differences between RPs and CPs in the number of MTGCs per standardised length. Pearson's  $\chi^2$  test was used to test observed differences in frequencies between groups. Significance was defined as  $p < 0.05$  for all tests.

### RESULTS

All RPs were clinically diagnosed as PA. Histologically, 22 of 23 cases (96%) were PA. In one case (4%) the basal decidua was not intact, implying that histologically it should be considered placenta accreta.

The obstetric history showed that 5 of 11 (45%) of parous women with RP had previously experienced RP, compared with 0 of 6 (0%) women in the CP group ( $p < 0.05$ , see table 1). For the other variables there were no significant differences between the two groups, notably not with respect to previous caesarean or curettage.

The histological data are shown in table 2. MTGCs were present in the basal decidua of 19 of 23 (83%) RPs and in all CPs. Quantitatively, the median number of MTGCs per standardised

**Table 2** The number of multinucleated trophoblastic giant cells, defects in basal decidua and myometrial fibre attachment in control placentas and retained placentas

	Control placenta (n = 10)	Retained placenta (n = 23)	p Value
MTGCs (number per length of deciduas), median (range)	1.11 (0.94–3.75)	0.23 (0–1.26)	<0.001*
Basal decidua defects, n (%)	0 (0)	1 (4)	NS
Myometrial fibre attachment			
Present in any of the slides, n (%)	0 (0)	18 (78)	<0.001*
Present in percentage of slides, % (range)	NA	48 (25–100)	NA

\*Mann–Whitney U test (two-tailed).

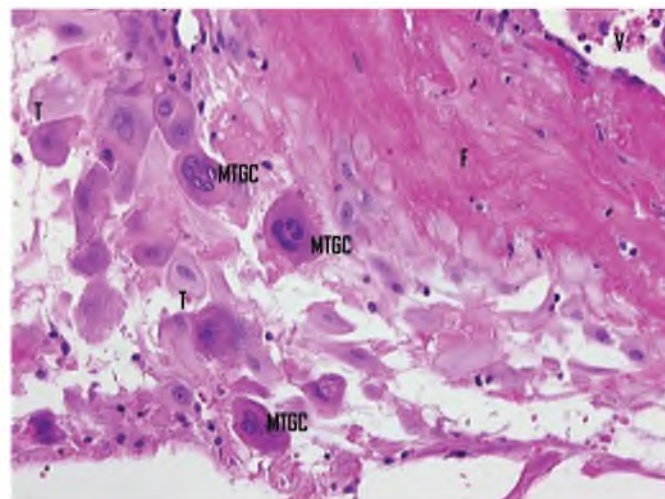
NA, not applicable; NS, not significant.

length of decidua basalis was significantly lower for RPs (0.23) compared with CPs (1.11). Figure 1 shows an example of MTGCs in the decidua basalis.

The basal decidua was intact in virtually all RPs (96%) and CPs (100%), whereas myometrial fibre attachment to the basal decidua was common in RPs (78%) in contrast to CPs (0%). Fig 2 depicts an example of RP with myometrial fibres attached to the intact decidua basalis.

### DISCUSSION

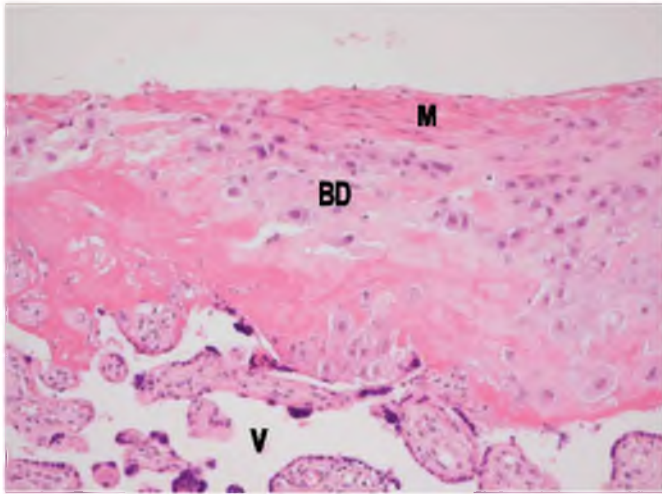
As to the cause of abnormal placental adhesion in PA, defects in the basal decidua are unlikely to be a cause because they were absent in 96% of our cases. It is also unlikely that insufficient uterine contractions were the primary cause of retainment as in all our patients active management of the third stage of labour included the use of uterotonics.<sup>17</sup> Our study suggests that abnormally strong attachment is the more likely mechanism by which placentas are abnormally adhesive, as myometrial fibres were found attached to the otherwise intact basal decidua in 78% of RPs in contrast to 0% of CPs. The latter is in line with previous studies showing that myometrial attachment is an abnormal finding with an incidence in randomly examined,



**Figure 1** Multinucleated trophoblastic giant cells (MTGCs) in the decidua basalis (H&E,  $\times 200$ ). At the light microscopic level, MTGCs are large cells (compared with the adjacent trophoblastic cells) containing two or more nuclei enclosed in a voluminous cytoplasm.<sup>14</sup> F, fibrinoid layer; V, villi; T, trophoblast cell.



## Original article



**Figure 2** Myometrial fibres attached to the intact decidua basalis in a retained placenta (H&E,  $\times 100$ ). V, villi; BD, basal decidua; M, myometrium fibres.

normally expelled, term placentas of 0.2%<sup>19</sup> to 0.9%.<sup>20</sup> Myometrial attachment in term placentas, as in our study group, must be regarded as a diagnostic sign of abnormal adherence.

Myometrial attachment was absent in 22% of our cases of PA. Although one might argue that abnormal adhesion did not exist in these cases, it seems more likely that it reflects that abnormal myometrial attachment in placenta adhesiva is a focal problem, as well as the fact that our way of sampling (four samples of each placenta) was unable to detect the focal problems in all placentas studied. Histochemical staining with, for example,  $\alpha$ SM1 (an anti-smooth muscle antibody), could have increased the absolute numbers of myometrium-positive slides, because small myometrial fibres are more easily detected in smooth-muscle staining.

Defects in the basal decidua were present in 4% of our cases of RP. Although defects in the basal decidua are a focal problem, it seems unlikely that this was caused by our sampling technique, as two previous studies using comparable sampling technique reported focally absent decidua in 100% of cases of placenta accreta.<sup>8,9</sup> We feel that our one case of RP with a defect in the basal decidua, and which was clinically diagnosed as PA, should be histologically classified as placenta accreta.

Frank and Kaufmann hypothesised that fusion of trophoblastic cells into MTGCs limits their invasiveness.<sup>15</sup> Our finding of a significantly reduced presence of MTGCs in the basal decidua of abnormally adhesive placentas compared with

controls seems to support this hypothesis. However, it does not answer the basic question of whether PA is caused by defective fusion of trophoblast cells into MTGCs in the decidua.

Our study does not provide information on the presence of MTGCs in the deeper layers of the uterus, as we did not take placental bed biopsies. Three previous studies have focused on these deeper layers. One study reported the expected presence of only a few MTGCs in the decidua and myometrium of hysterectomy specimens of placenta creta,<sup>9</sup> while the two other studies found no significant difference in the number of MTGCs in the myometrium of hysterectomy specimens between placenta creta and controls.<sup>6,13</sup> We conclude that although we did not study the MTGCs in the placenta bed and myometrium, in the literature there are no suggestions that fusion of trophoblastic cells in MTGCs takes place in deeper layers of the myometrium in PA compared with CPs.

The cause of defective fusion of trophoblast cells into MTGCs is unknown. Theoretically, the fusion could be impaired by dysregulation through decidua-derived factors. Uterine natural killer cells in the maternal basal decidua have the ability to secrete several cytokines, chemokines and angiogenesis-regulating molecules that regulate trophoblast inhibition and proliferation.<sup>21–23</sup> It is conceivable that these factors play a role. More research is needed to disclose the role of deciduas-derived factors in RP. Recent studies have suggested that in placenta accreta defective fusion of trophoblast cells into MTGCs is associated with excessive numbers of mononuclear trophoblast cells.<sup>13,24</sup> We intend to address these issues in further studies of PA.

In conclusion, RP caused by PA is associated with a reduced number of MTGCs in the basal decidua and not by defects in the basal decidua. We speculate that these findings may indicate that defective fusion of trophoblastic cells into MTGCs plays a causative role in PA.

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**Competing interests:** None.

**Ethics approval:** Ethics approval was obtained from the Radboud University Medical Ethics Committee.

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## Take-home messages

- Placenta adhesiva is not associated with defects in the basal decidua, in contrast to placenta accreta.
- Placenta adhesiva is associated with the presence of myometrial fibres attached to the basal decidua. This may be regarded as a diagnostic sign of abnormal attachment of the placenta to the uterine wall.
- Placenta adhesiva is associated with a reduced amount of multinucleated trophoblastic giant cells (MTGCs) in the decidua basalis. This may suggest that defective fusion of extravillous trophoblast cells in MTGCs could play a causative role in this condition.

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## PANCREATIC PATHOLOGY MEETING

**Wednesday 2nd December 2009**

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This one-day meeting will focus on the reporting of pancreatic cancer specimens (including a live specimen dissection session) in the light of the revised RCPATH Minimum Dataset that will be issued later this year. Further topics included in the programme are endocrine pancreatic tumours and their differentials, pancreatobiliary cytology, and discussion of training and research in pancreatic pathology in the UK. The meeting concludes with an Open Forum session, in which difficult cases will be discussed in a multidisciplinary approach. 5 CPD credits have been awarded. Target audience: specialist pancreatic pathologists, consultants with an interest in pancreatic pathology, senior trainees in pathology and oncology, members of the pancreatic MDTeam. The meeting precedes the Annual Scientific Conference of the Pancreatic Society of GB & Ireland, 3–4. December 2009, Weetwood Hall, Leeds. The conference programme is multi-disciplinary and an impressive faculty of national and international speakers will address clinical, pathology, training and research issues in pancreatic cancer.

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